

# **Guidelines for the Prevention and Control of Tuberculosis in Nonhuman Primates**

## **I. Introduction**

Because tuberculosis is a zoonotic disease that can be devastating and terminal in nonhuman primates (NHPs), and may be transmitted from humans to NHPs, it is necessary to establish guidelines for the prevention and control of this pathogen within the NIH intramural research program. These guidelines apply to all NIH-operated facilities.

## **II. Prevention**

Preventive measures are required to protect NHPs and personnel who come into contact with NHPs that may be harboring *Mycobacterium tuberculosis* complex.

### **A. Quarantine**

The entry of NHPs into NIH operated facilities must be in compliance with the NIH NHP Quarantine Policy, Policy Manual 3044-1, "Nonhuman Primate Quarantine." Contact the NIH Animal Center, Division of Veterinary Resources (DVR), Office of Research Services (ORS) (301-435-4056) for further information.

### **B. Husbandry Practices**

The animal husbandry and sanitation practices as applied to NHPs at the NIH are designed to prevent the spread of pathogens including tubercle bacilli. To this end, tuberculocidal detergent disinfectants (the label must read tuberculocidal) must be used in facilities housing NHPs. Cleaning equipment must remain in one room unless it is effectively disinfected between rooms. Sanitation schedules and practices must be in compliance with all applicable regulations, policies and guidelines.

NHP holding and procedures rooms must be under negative pressure relative to adjacent corridors. Husbandry practices must minimize the production of aerosols in animal rooms, e.g., sanitizing room surfaces and sanitizing animal cages and litter pans or trays. Other procedures, including research procedures, must be carried out in a manner to prevent the generation of aerosols that potentially contain pathogens. High pressure washing of cages and room surfaces can be performed only after the NHPs have been removed from the room and with proper protection of personnel including protection from splash.

### **C. Monitoring Procedures**

- 1. Tuberculin Skin Testing** - Tuberculin skin testing (TST) is the primary tool used to detect tuberculosis in NHPs.
  - a) Methods:** Using a sterile needle for each NHP, inject 0.1 ml. of Mammalian Tuberculin intradermally into one eyelid near the edge or into the abdominal skin or both; 0.05 ml. can be used in small NHPs, e.g., some New World species. Usually the eyelid is preferred as it is relatively easy to observe. If the abdomen

is used, the hair should be clipped without traumatizing the skin and the injection site noted. The abdominal skin test is most commonly used when retesting suspect NHPs. The advantage of using the abdomen is that any induration can be measured and a saline control injection can be used.

**b) Reading TST:** Observe the animals for reactions at 24, 48, and 72 hours post-injection under good lighting conditions. The readings must be made by a trained technician. Any reactions or suspected reactions are to be observed and interpreted by the attending veterinarian. The following grading systems should be used:

**(1) Eyelid injections:** When using the following grading system, the actual descriptions or corresponding reaction grade should be entered into the animals record.<sup>1</sup>

#### **Reaction Grade Description of Changes:**

**0** - No reaction

**1** - Bruise - extravasation of blood in the eyelid associated with the injection of tuberculin.

**2** - Varying degrees of erythema of the palpebrum with minimal swelling.

**3** - Moderate swelling with or without erythema.

**4** - Obvious swelling of the palpebrum with drooping and varying degrees of erythema.

**5** - Marked swelling with necrosis and eyelid closed or partially closed.

Grades 0, 1 and 2 are considered negative, grade 3 is suspect and grades 4 and 5 are considered positive.

**(2) Abdominal injections:**

<b>Induration at widest point</b>	<b>Interpretation</b>
< 5 m.m. 5 to 10 m.m.	Negative Suspect
> 10 m.m.	Positive

**c) Frequency of TST:** The following intervals for TST of species or groups of NHPs is recommended during post quarantine holding. Because of a number of

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<sup>1</sup> Modified from Fox JG, Anderson LC, Loew FM, Quimby FC, eds. Laboratory Animal Medicine, 2<sup>nd</sup> ed. Academic Press, Inc., Orlando FL, 2002.

variables, the facility veterinarian may elect to TST at less frequent intervals. When NHPs are TST at less frequent intervals than these recommendations, the facility veterinarian who is to receive any of those NHPs must be notified of that fact before the animals are transferred.

Species or Group	Recommended TST Schedule
New World Monkeys	Semiannually
Macaque species	Quarterly
Baboons	Semiannually
Chimpanzees	Annually
African Monkeys	Quarterly
Prosimians	Semiannually

- d) Anergic NHPs:** Tuberculous NHPs infrequently become anergic to TST. Tuberculosis should be considered and further testing performed on animals that have unexplained weight loss or non-healing wounds. Additional testing may include: cytology and culture swabs of non-healing wounds, chest radiographs, acid fast bacillus smear, culture and PCR (polymerase chain reaction) of gastric and/or bronchial lavage, PCR of feces or tissues, and other methods as they are validated.
- e) Suspect NHPs:** Tuberculosis should be considered and further testing performed on animals with a suspect response on palpebral or abdominal tests. Additional testing may include: testing the contralateral eyelid, performing an abdominal test if not already performed, chest radiographs, acid fast bacillus smear, culture and PCR (polymerase chain reaction) of gastric and/or bronchial lavage, PCR of feces or tissues, and other methods as they are validated.
- f) Sensitized Nontuberculous NHPs:** NHPs become reactive to TST when injected with immunologic materials that contain Complete Freund's Adjuvant (CFA) because it contains cell walls of tubercle bacilli. When feasible, other adjuvants should be used to avoid ameliorating the usefulness of the best test available for monitoring NHPs for tuberculosis. If it is necessary to use CFA, the NHP(s) is to be tuberculin tested the week before the CFA is injected. If the NHP is euthanatized before its next tuberculin test is scheduled, there are no additional requirements. Otherwise, the NHP is to be weighed monthly to detect any weight loss, and, at the time the NHP would normally be tuberculin tested, other examinations are to be performed for the detection of tuberculosis. [Such testing may include polymerase chain reaction (PCR) and/or acid fast bacillus smears and cultures of fecal and/or gastric washings for mycobacterium species.] If tuberculosis is confirmed in other NHPs in the holding room housing a CFA exposed NHP, the potentially exposed NHP(s) that previously received CFA should be euthanatized.

## **2. Necropsy**

All NHPs that die or are euthanatized will be necropsied by the Pathology Section, Diagnostic and Research Services Branch, DVR, ORS. If there is a need, for research reasons, to have a NHP necropsied at a collaborating institution, a complete necropsy is to be performed by a veterinary pathologist and a copy of the findings are to be sent to the Chief, Pathology Section, Diagnostic and Research Services Branch, DVR, ORS.

These necropsies may be performed by other qualified veterinarians at NIH operated facilities that are more than 100 miles from the Bethesda campus, e.g., Rocky Mountain Laboratories and NIEHS.

## **3. Radiographs**

Chest radiographs may be used as an additional test procedure but cannot be used as the only screening procedure. Chest radiographs can be difficult to interpret especially in macaque species.

## **4. Protection of NHPs from Personnel**

The procedures mandated in Policy Manual 3044-2, Protection of NIH Personnel Who Work with Nonhuman Primates (PM 3044-2), to protect personnel from the zoonotic diseases of NHP, also protect NHPs from being exposed to tubercle bacilli from humans.

### **III. Protection of Personnel**

PM 3044-2 addresses personnel protection and is to be followed including specific mucous membrane protection procedures addressed in SOPs at each facility. PM 3044-2 states that NIH employees, special volunteers, guest workers, visitors and contract personnel shall comply with procedures set forth in this policy.

All NIH personnel who work with or around NHPs are required to participate in the Animal Exposure Surveillance Program (AESP); contract personnel are required to participate in an equivalent program. Transient visitors who are required to enter a room housing NHPs, but do not have direct contact with the NHPs, are not required to participate in the AESP but are to wear a single-use dust/mist mask in addition to other required protective clothing (see PM 3044-2). Additionally, transient visitors must be free of signs of active disease.

Biosafety precautions must be taken when dealing with a diagnosed tuberculous NHP, a NHP that is a tuberculosis suspect, and when collecting and handling samples to be cultured for tubercle bacilli.

### **IV. Handling Tuberculous NHPs**

#### **A. Immediate Euthanasia**

When a clinical diagnosis of tuberculosis is made in a NHP, it is immediately euthanized (unless IV. B. or C. below applies) and the carcass is taken to the Pathology Section, Diagnostic and Research Services, DVR, ORS for necropsy, or other facilities as discussed under paragraph II.2 above, and the Occupational Medical Service (OMS), Division of Occupational Health and Safety is notified. The cage and room where the tuberculous NHP(s) was held are sanitized and the remaining NHP(s) are placed under quarantine.

Quarantine means:

- 1) access to the room is limited to a few essential personnel,
- 2) protective clothing (Tyvek<sup>®</sup> jump suit, shoe covers, head bonnet, mask, latex, nitrile, vinyl or rubber gloves and eye protection) is worn in the room and is not removed from the room except to be autoclaved,
- 3) NHPs are not placed in or removed from the room, and
- 4) the NHPs in the room are tuberculin tested every two weeks until five tests have been performed with negative reactions; the first of these tests is administered about one week after the test that identified the tuberculous NHP.

When 5 tests have been administered with negative reactions, the quarantine may be terminated, except that NHPs are not placed in or removed from the room until a tuberculin test is administered four weeks after the last of the 5 tests with negative reactions being observed. A diligent effort will be made to locate all NHPs that were housed within the last 60 days in the room in which the tuberculous NHP was housed. These NHPs will be tuberculin tested on the same schedule as the NHPs currently housed in the quarantined room.

## **B. Delayed Euthanasia**

The euthanasia of a tuberculous NHP can be delayed if the animal is of great value to a research project and can be isolated to minimize the spread of tubercle bacilli to other NHPs or humans. The room in which such a NHP was held when the clinical diagnosis was made will be placed under quarantine as described in IV. A. above. The following individuals and organizations must be notified and agree on how such a NHP will be handled before the euthanasia can be delayed: the facility veterinarian, the Animal Program Director of the owning institute, and the Division of Occupational Health and Safety. When a decision to delay the euthanasia has been agreed upon by the above parties, the Occupational Medical Services of the Division of Occupational Health and Safety must be notified.

## **C. Treatment of Tuberculous NHPs**

Valuable NHPs of those species that are not highly susceptible to tuberculosis may be treated to free them of tubercle bacilli, e.g., chimpanzees. A multiple drug regimen based on the most current practice standard must be used in the treatment and the treatment must be for at least 6 months. The same isolation, notification and agreements must be performed as noted in IV. B. above before extended holding and therapy can be instituted.

## V. Records

It is important that each NHP 's tuberculin test be accurately entered into its clinical record and that this record include where the animal has been housed including dates. Accurate records are also important in detecting unexplained weight loss or non-healing wounds which may be indications of tuberculosis in NHPs.

## VI. References

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2. NIH Manual 3040-2, Animal Care and Use In the Intramural Program.
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